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IMMUNORADIOMETRIC ASSAY (IRMA) FOR THE DETECTION OF E.COLI PROTEINS IN RECOMBINANT HUMAN GROWTH HORMONE PREPARED AT IPEN-CNEN/SP

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With the introduction of recombinant pharmaceuticals produced in E. coli cells a sensitive and objective evaluation of E. coli protein (ECP) content has become necessary as an indicator of final purity. In order to eliminate any possible adverse effect, a limit of less than 10 ppm (w/w) is presently accepted by the authorities, being therefore necessary a sensitivity of at least 5-10 ng of ECP/ml. Such sensitivity can be obtained neither with silver stained SDS-PAGE nor with immunoblotting techniques, while enough precision and accuracy can only be obtained setting up an assay that is specific for the particular bacterial strain and purification process being used.

For these reasons we set up an IRMA for ECPs derived from our RRI cells, containing the expression vector but not the human growth hormone (hGH) gene. ECPs submitted to a partial purification process and possibly contaminating the hGH peak were used for the immunization of four female New Zealand white rabbits during about three months. The antisera so obtained were cheked via a simple RIA and those with the highest titers were purified on an affinity chromatography column, obtained through covalently coupling of the same ECP to CNBr-activated Sepharose 4B. The purified anti-ECP gamma globulins were labelled with ^{125}I and used in a sandwich type assay on microtiter plates, adsorbed with the same antibody. Sensitivities of the order of 0.5 - 1 ng/ml together with a satisfactory intra-assay precision (CV= 4-10%) were obtained.